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IN VITRO IXODICIDAL ACTION OF NEEM (*AZADIRACHTA INDICA* A. JUSS) ON *RHIPICEPHALUS MICROPLUS* ENGORGED FEMALE TICKS

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ABSTRACT

We evaluate the mortality of *R. microplus* engorged female ticks exposed to vegetal extract of *A. indica*. Fifteen ticks were selected for each and assigned into four treatments: T1-T4. The specimens of each group were immersed for a period of 15 minutes in its respective solution. Once the time was completed, they were removed from the container, the excess product was removed with blotting paper, and placed in Petri dishes. The specimens were left to rest inside an extraction hood at 31.6 °C and 56% relative humidity and were observed every 24 hours until death. The highest mortality rates were observed in the T3 (100%), while in the T2 and T4 (93%) and in the T1 had a mortality of 73%, at 72 h post-treatment. It is recommended to carry out more *in vitro* studies with neem alone or in combination with other plant extracts.

Keywords: Mortality,ticks,neemextract.

INTRODUCTION

Ticks (Acari: Ixodidae) are obligate bloodsucking ectoparasites of domestic and wild animals (Uspensky, 2017). These external parasites cause the most economic losses in livestock production around the world (Domínguez-García et al., 2016). With their bites cause irritation, discomfort, and stress, which interferes with the animal feeding, resulting in a decrease in meat production, milk production and delayed growth (Rodriguez-Vivas et al., 2018). Furthermore, during the blood feeding ticks can act as vectors of medically important pathogens (Kasaija et al., 2021). Anaplasmosis, Babesiosis, and Theileriosis are some of the most important diseases, which affects the blood-lymphatic system, causing fever, anemia, dyspnea, weight loss, milk drop, and even death (Nejash, 2016). The mentioned reasons make it especially important to investigate for alternatives to control infestations by these ectoparasites.

As described by Ostfeld et al. (2006) and Vudriko et al. (2018), chemical acaricides are the main method for tick control; unfortunately, the indiscriminate and frequent use of these products leads to the development of resistant strains, secondary effects on the environment, and the presence of chemical residues in products and by-products of animal origin. As revievew by Showler & Saelao (2022), some investigations have been done looking for different ways to control arthropods of veterinary economic importance, highlighting the search and use of natural plant products. Essential oils have proved acaricidal (Luns et al., 2021, Wanzala et al., 2014) or repellent activity against ticks. Selles et al. (2021) argue that natural compounds are safe to humans, animals, and environment due to their solubility in water and low toxicity, as consequence contributes to the production of milk and meat free from dangerous chemicals. Neem (*Azadirachta indica*) belongs to Meliaceae family, and is well known as a versatile plant having a wide spectrum of biological activity, including acaricidal properties of its extracts (). In this study we evaluated the acaricide effect of neem extract on *R. microplus* engorged female ticks.

MATERIALS AND METHODS

Tick collection and Experiment

The *R. microplus* engorged adult females were collected in a farm located in Cuajinicuilapa, Guerrero, México. The production unit has small livestock, dedicated to the breeding, milking and sale of bovines. The experiment was carried out in the Multidisciplinary Laboratory of the Faculty of Veterinary Medicine and Zootechnics No. 2 of the Autonomous University of Guerrero, at the same mentioned city. The municipality is located between 16°28'34" N and -98°25'46" W, at an altitude of 46 masl. The temperature has a range of 24 - 26°C,

with an annual rainfall of 1,100 - 1,300 mm, with a warm sub-humid climate with rains in summer, with an average humidity of 97% (INEGI, 2010). The ticks were maintained in a room under controlled conditions: 31.6 °C, 56 % humidity, and 12-h photoperiod).

Neem extract (Adbaneem Plus S-408®)

Adbaneem Plus S-408® - ADVAM is a natural commercial agricultural adjuvant, as declared in the technical data sheet, the product contains oil neem extract. As physical properties it has a brown liquid texture with a slight smell of garlic and bitter taste. Its melting point is 12.7° degrees, and it boils to 200° degrees, is slightly soluble in water and degrades rapidly in the environment.

Treatments

In brief, 2.5, 5.0, 7.5, and 10.0 mL of product were diluted in 7.5, 5.0, 2.5, and 0.0 mL, of distilled water dilutions for a final volume of 10 mL. For each treatment, the quantity was measured with sterile plastic syringes (Plastipak® - 10 mL) and placed in plastic labeled disposable cups (Plastic World® - 100 mL). The groups were assigned as follows: T1 = 25% product (P) - 75% distilled water (Dw), T2 = 50% P - 50% Dw, T3 = 75% P - 25 % ml Dw, and T4 = 100% P.

Tick selection

The ticks were selected and classified according to their physical appearance, size, and mobility. All ticks that did not show signs of mobility or were weak, that their size was very different or that showed any physical damage that could modify the results, were discarded. In total, 75 ticks were selected, and 15 individuals were assigned for each treatment group: T1-T4. The studies by Marín-Quintero & Vargas-García (2017) and Guerrero & Guerrero (2017) were taken as reference for sample calculation.

Experimental model

Each tick group were placed inside the containers with its respective dilution. Every group were left submerged for 15 minutes, and once completing the time, were extracted from the container. The excess of the solution was removed with blotting paper, and each tick was returned to their Petri dish. The observation of the specimens was carried at 24-hour intervals (Bravo et al., 2008).

Mortality measurement and Data analysis

To observe the treatment effect, the ticks were placed on sheets marked with red circles, expecting to observe mobility in a period of 10 minutes (Giannelli et al., 2012). The specimens were considered as dead when did not show movement of its legs (Guerrero & Guerrero, 2017). The mortality was measured as described previously (Marín-Quintero & Vargas-García, 2017). The results are presented as descriptive statistics and expressed as percentages.

RESULTS AND DISCUSSION

This study was performed to evaluate the *in vitro* mortality of *R. microplus* engorged ticks with a commercial oil neem extract. During the study, the neem extract showed an ixodicide effect over the ticks. The highest mortality rates were observed in T3 from day three, while in the T2 and T4 was observed from day four. All the specimens of the T3 and T4 dead on day five, while T1 and T2 on day six. At 72 h post-treatment the T1 had a mortality of 73%, while in the T2 and T4 the mortality of the specimens was 93%; however, in the T3 the mortality was of 100% at the same time. The number of dead specimens is presented with respect to the hours of treatment in the Figure 1.

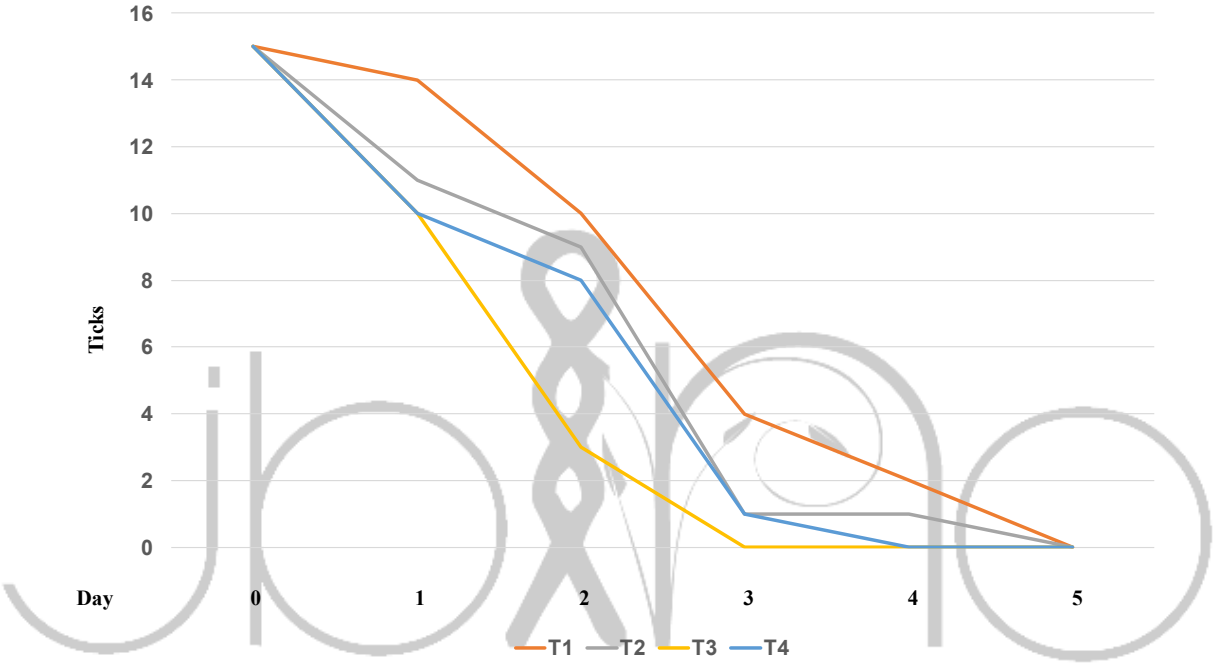

Figure 1. Tick mortality with respect to neem exposure time.

Gil et al. (2013) mentions that some substances present in plants act dissolving the lipids of the cuticle of the exoskeleton; while others generate paralysis and finally the death of the arthropods (Rodríguez et al., 2010). Secondary metabolites are chemicals produced by some species of plants for their defense, communication, and reproduction (Erb & Kliebenstein, 2020). Nearly 3,000 secondary metabolites of plant origin have been characterized with biological activity on different organisms (Iriarte-del Hoyo et al., 2012). These phytochemical compounds comprise a wide variety of chemical structures such as terpenoids, alkaloids, phenolic compounds, sulfur compounds, iridoids, and steroids (Divekar et al., 2022). In the United States, were carried out toxicological evaluations of a product Neem-based (Margosan-O®). In the study, no skin irritation reactions or irritation eye or signs of inhalation poisoning with this type of compounds were observed (Schmutterer, 1990).

Neem oil has been used against other tick species than *R. microplus*. Abdel-Shafy & Zayed (2002) reported that in *Hyalomma anatolicum excavatum* ticks, mortalities ranged from 68%, 74%, 82%, 88%, 100% on 1st, 2nd, 3rd, 7th, and 15th days post treatment, respectively. Tamirat et al., (2014) evaluated a commercial neem oil diluted with distilled water, reported an 8%, 17%, 25%, 33%, and 50% mortality in adult *Amblyomma cohaerens* ticks at 20%, 40%, 60%, 80%, and 100% concentration, respectively. Mahran et al. (2020) employed methanolic extract of neem leaves on adult *Hyalomma dromedarii* ticks, reporting mortalities ranging from 25%, 40%, 55%, 85% and 100% at 1st, 2nd, 3rd, 7th, and 15th days post treatment, respectively.

Among the studies performed to evaluate the efficacy of *A. indica* over *R. microplus* ticks, researchers reported variable results. Srivastava et al. (2008) observed mortality ranging from 15% to 50% in concentrations of 2-5%, in 6% concentration, 65%, while and in 7%, 8%, and 9% revealed a 70% of adult tick mortality at 24 h post treatment. Broglio-Micheletti et al. (2010) testing the action of oil neem in the control of *R. microplus* ticks in laboratory observed mortalities ranging from 48% - 96%, with two different concentrations of neem oil. Gigliotti et al. (2011) obtained an average effectiveness from 86%, 88%, 93% and 95%, when evaluated the *in vitro* acaricidal activity of neem against *Rhipicephalus microplus*. Santos et al. (2012) revealed an average efficiency of 74% of the effect of neem extract on *R. microplus* engorged females. Differences in the results may be due to extraction method, type of solvent used, phytochemical solubility, as well as impact of time and temperature maintained for the extraction of the extracts (Kumar-Das et al., 2020).

All treatments caused 100% mortality over the ticks subjected to the experiment, even in the lowest concentration solution. Neem oil extracts is a viable alternative for *R. microplus* engorged female ticks *in vitro*. It is necessary to carry out studies on its efficacy in dilutions of less than 25%, and to prove the efficacy of the product *in vivo*. Tick mortality showed important effects, in this sense, the evaluation of the product is required to determine the potential of its ovicidal and larvicidal capacity.

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